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UNVEILING THE EFFECTS OF DROUGHT STRESS ON MAIZE HYBRIDS: CHANGES IN CHLOROPHYLL CONTENT, MORPHOLOGICAL TRAITS AND SOIL-PLANT WATER DYNAMICS

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Abstract

This experiment aimed to assess chlorophyll content under drought conditions in two maize hybrids, KWS Inteligens and Kashmir, within microcosms. The parameters analyzed included chlorophyll content, soil moisture, morphological and growth characteristics based on the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie), leaf area, final dry biomass and soil-plant water budget. Chlorophyll content and soil moisture were monitored twice weekly for normal hydric regime (RHN) and for drought or hydric stress (SH). Among the findings, Kashmir hybrid recorded the highest average chlorophyll content of 170 SPAD units after 20 days under RHN, while Inteligens hybrid showed the greatest increase in leaf area of about 2.24 dm². In case of dry biomass accumulated by Kashmir hybrid, a reduction of 63% was achieved under drought compared with RHN, while for Inger this percent was higher with 3%. The results highlight that both hybrids endured drought conditions for up to 23 days from emergence to wilting.

***Key words**: BBCH scale, soil-plant water budget, KWS Inteligens, KWS Kashmir, water stress.*

INTRODUCTION

In Romania, maize is the most extensively cultivated crop (Popescu, 2018). As one of the most important cereals for both human and animal consumption, it is primarily grown for grain and animal feed (Dinca et al., 2020). Within the European Community, Romania holds 30% of the total area cultivated with maize. According to FAOSTAT statistics for 2019, Romania had 2,691,930 hectares of maize cultivation, with an average yield of approximately 6.5 t ha^{-1} (64,999 hg ha^{-1}), and a total production of 17,432,220 tons per year (FAOSTAT, 2019). Each year, at the regional scale the frequency and intensity of droughts are increasing (Mustafa et al., 2023). In general plants fresh biomass comprise between 85-90% water for a functional metabolism and assuring integrity (Luqman et al., 2023). These data highlight the significance of maize cultivation and demonstrate its suitability for the eco-pedo-climatic conditions in Romania. Over time, maize has become one of the most widespread and adaptable agricultural crops globally (Ranum et al., 2014). Today, maize is cultivated across the entire globe, from tropical plains to temperate regions and even at high altitudes. The crop's characteristics are closely tied to temperature and precipitation during the growing season (Vâtcă et al., 2021). Climate change has emerged as the greatest global challenge, posing threats particularly to crop patterns and yields (Vâtcă et al., 2021), thereby endangering food security for a growing population (Bennetzen et al., 2016; Godfray et al., 2010). According to the fifth assessment report of the Intergovernmental Panel on Climate Change, climatic conditions are expected to alter the spatial distribution of crops worldwide (Kogo et al., 2019).

Establishing climate risks for individual crops on a global scale and their future evolution is a key requirement for developing a resilient and robust food production system that ensures food security (FAO, 2001; Shirley et al., 2020). Beyond its economic importance, maize plays a crucial role in human nutrition and global food security (Tanumihardjo et al., 2020). As a rich source of carbohydrates, fiber, vitamins, and minerals, maize is consumed in a wide variety of forms, from fresh kernels to processed products (Ranum et al., 2014).

In many countries, maize is a staple food for millions of people, underscoring its importance in providing sustenance for vulnerable communities (World Health Organization, 2020). One of the greatest challenges facing contemporary agriculture is water stress (Rosegrant et al., 2009), a phenomenon exacerbated by climate change and intensive exploitation of natural resources (Maja and Ayano, 2021). In this context, maize, as a fundamental agricultural crop, is subjected to significant pressures, being at risk of yield deterioration and productivity decline under water scarcity conditions.

Assessing chlorophyll content in maize under such conditions is thus essential for understanding plant adaptation mechanisms to water stress and developing agricultural management strategies that ensure crop sustainability and resilience.

Chlorophyll is the pigment responsible for photosynthesis, the vital process through which plants convert sunlight into chemical energy (Mandal and Dutta, 2020). Under water stress conditions, chlorophyll content may be affected, and evaluating this aspect can provide valuable insights into plant health and adaptability to water-deficient environments (Rosero et al., 2020; Pour-Aboughadareh et al., 2019; Qiao et al., 2024). Some results are contradictory regarding the effect of water stress on chlorophyll content. Findings declared decreased values of chlorophyll contend under drought (Khayatnezhad & Gholamin, 2012; Pour-Aboughadareh et al., 2019; Qiao et al., 2024) or increased values (Rosero et al., 2020). When the values of chlorophyll content are increased under drought stress conditions results that the maize hybrids are resistant to drought conditions (Khayatnezhad & Gholamin, 2012).

The general aim of the study was established to highlight the effects of drought on two KWS maize hybrids. Adhesive objectives were represented by the assessment of (i) experimental factors hybrid, water regime, assessment date effect on chlorophyll content dynamic; (ii) the influence of soil water pool upon chlorophyll content values of both maize hybrids (iii) highlighting the soil water budget in different time-assessments within the experimentation; (iv) correlation coefficient among all parameters from the experiment and (v) providing recommendations regarding maize hybrid best performance under drought.

MATERIALS AND METHODS

Experimental set-up: The experimental protocol consists of 4 treatments in three replicates, therefore each hybrid tested was subjected to normal water regime and also to water stress (drought) in controlled conditions. Thus, T1-T3 included the replications for KWS Inteligens maize hybrid in a normal water regime, T4-T6 the KWS Inteligens hybrid in water stress, T7-T9 the KWS Kashmir hybrid in a normal water regime and T10-T12 the KWS Kashmir hybrid in water stress (Figure 1). The microcosms selected for the experiment had a diameter of 15 cm, and a height of 11 cm.

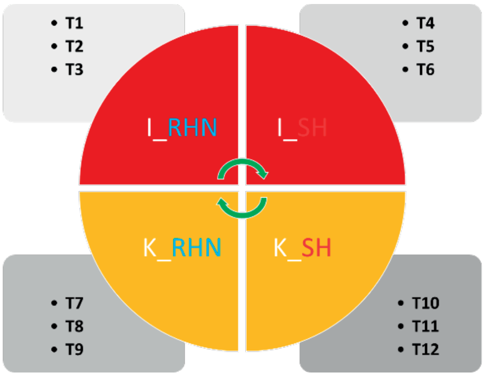

Figure 1. Experimental design. Note: T1-12 - experimental treatments (with replications), RHN - normal hydric regime, SH-hidric stress, drought, I - maize hybrid Inteligens, K - maize hybrid Kashmir

Growth substrate: In each microcosm 500 g soil was introduced. The growth substrate used was a universal soil with a chemical composition as follows: nitrogen (N) 225 mg/l, phosphate (P_2O_5) 125 mg/l, potassium oxide (K_2O) 125 mg/l, with a pH of 7.5, KCl salts below 3 g/l and 57% organic matter, with moisture content of 60%. The RHN was maintained by adding weekly an amount of 200 ml tap water.

Features of maize seeds and tested hybrids: Maize seeds tested were aquired from the Turda Agricultural Research and Development

Station (ARDS Turda - https://scdaturda.ro/). The KWS Inteligens maize hybrid, classified as a PLUS4GRAIN hybrid from the FAO 420 group (mid-late) it is recommended for early sowing. The high production potential is supported by an erect foliage and an excellent ability to stay green, which contributes to efficient photosynthesis and the accumulation of nutrients in the grains. The KWS Kashmir maize hybrid is part of the FAO 370 group, being a mid-early, simple hybrid. Adaptability to various climate and soil conditions is a defining feature of the KWS Kashmir hybrid. One of the main advantages of the KWS Kashmir hybrid is its high production potential. Adaptability to various climate and soil conditions represents also a defining feature of the KWS Kashmir hybrid. The seeds of maize KWS hybrid Kashmir had at the begging of the experiment the average large diameter of 1.15±0.06 cm (SE) and small diameter of 0.67±0.06 cm. The seeds of maize KWS hybrid Inteligens had at the begging of the experiment the average large diameter of 1.15±0.12 cm and small diameter of 1.00±0.61 cm. A number of 10 seeds were placed in each microcosm. Field density recommendations for both maize hybrids are depent on environmental conditions: 65,000 plants/ha in arid areas and 70,000 plants/ha in favourable areas.

Assessments during the experimental trial: Plant physiological monitoring was performed every 2 weeks, from germination to wilting, consisting of measuring the chlorophyll content from the leaves using the MC-100-Apogee Instruments chlorophyll meter (Zlatev et al., 2023). The percentage of soil moisture was done at the time of chlorophyll content readings using the SM-150-Soil moisture kit hygrometer.

Leaf area was measured according the Caliper method and calculated following Anikeev-Kutuzov formula (Nasiyev et al., 2022). Plant growth parameters were identified according to the BBCH phenological scale (Meier, 2018). Plant growth evolution was performed twice a week, administering 200 ml of water only to hybrids under normal water regime, to test the growth and development capacity of the other plants subjected to water stress under drought conditions. At the end of the experiment, the green aboveground biomass was sampled from each microcosm in aluminium containers to determine the plants dry biomass after drying in an oven at 105°C for 24 h until constant weight (Portillo-Estrada et al., 2015). Data analysis: Statistics on the entire dataset were obtained with the open-source RStudio program (R Core Team, 2020), with the package "psych for obtaining and extracting the basic statistics values (Corcoz et al., 2022; Revelle, 2017; Stoian et al., 2022). The package "agricolae" (de Mendiburu, 2019) and "broom" (Robinson, 2014) was used to extract and export three-way (*hypothesis (i)*) and two-way (*hypothesis (ii-iii)*) ANOVA and Least Significant Differences (LSD) test (Stoian et al., 2016; Sângeorzan et al., 2024). For the extraction of correlogram (*hypothesis (iv)*) between all obtained parameters, the package "corrplot" (Wei, 2017) was used. To analyze the effect of treatments on chlorophyll, following *hypothesis (ii)*, boxplots were created using the online platform - https://www.statskingdom.com/, by selecting the advanced box plot graph where are visible all the means, medians, quartile 1, quartile 3, the minimum and maximum values obtained from all repetition assessed.

The pye chart figures were selected to represent all five BBCH scale assessment based on evolution under tested water regimes. The big and first pye chart were chosen to represent the total average percent of germinated seeds from each of the four treatments. Different BBCH sub-stades are projected in the right wit and present the corressponding average percentages for all treatments in evotution. Following all statistical values, figures and correlations the *hypothesis (v)* is accomplished as a conclusion of the study. The formulas used in the experiment:

Maize plants moisture content at the end of experiment – MWC = (FB-DB)/DB*100, where: MWC = maximum plant moisture content at the end on the experiment (Sala et al., 2007), FB = fresh biomass, DB = dry biomass;

Soil moisture content at the end of the experiment – SWC = (HS-DS)/DS*100, where: SWC = soil moisture content, HS = humid soil, DS = dry soil. The mass of dry soil material was considered as a reference state (Yolcubal et al., 2004).

RESULTS AND DISCUSSIONS

Significant differences (*p<0.001*) were seen between the individual experimental factors - water regime treatments - and different assessments dates (Table 1). Also, the interaction between water regime and different assessment dated had significantly influence the results presenting leaf chlorophyll content and soil moisture content. The triple interaction of all three factors analysed together did not show a significant effect on the obtained results.

Table 1. Three way ANOVA of experimental factors and their impact on the differences between chlorophyll content (C) and soil moisture content (SH)

	C		SH	
	F	*p val*	F	*p val*
H	2.68	0.103	0.57	0.453
W	47.47	<0.001	297.21	<0.001
A	155.99	<0.001	18.31	<0.001
H:W	0.55	0.458	1.23	0.269
H:A	0.18	0.668	0.07	0.795
W:A	27.07	<0.001	12.24	0.001
H:W:A	0.29	0.591	0.33	0.566

Legend: H - maize hybrid; W - water regime;A - assessment date.

The chlorophyll content of maize hybrid leaves is direct dependent on soil moisture content (Figure 2). The highest average value of 202 SPAD units was observed at the maize hybrids from drought (SH) in the second assessment conditions. This value was significantly higher compared to RHN from the same date and also when compared with almost all treatments and assessment dates except first assessment from RHN and third assessment of SH and RHN. The chlorophyll content average values were in the range of 144-178 SPAD units in case of assessment 2-5 from RHN treatments.

Soil moisture content was highest at the beginning of the experiment in RHN treatment, especially at first assessment (Figure 3). Because of drought conditions simulated in SH treatments, the most lower values were observed at the assessments 5 and 6. These values were significantly lower compared to the percentages of soil moisture content from the treatments SH on the assessment dates range between 2-4. Given the fact that at the beginning of the experiment the soil moisture content started from 60%, the SH_1 from first assessment was grouped with the treatments RHN 2, 3 and 5. Germination in the field is very well represented by the results from this experiment in controlled conditions.

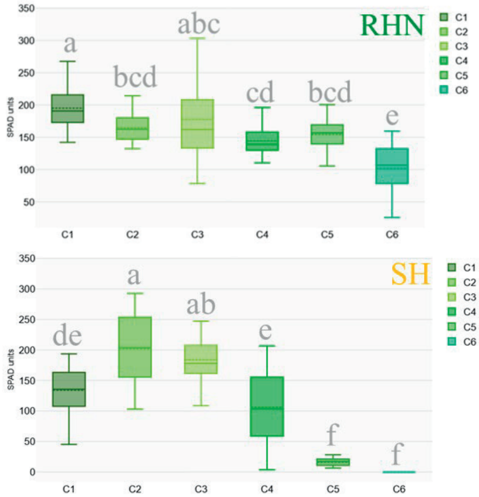

Figure 2. Differences induced by water regime on chlorophyll content values recorded in six assessment dates C1-C6. RHN-normal hydric regime and SH-hydric stress-drought. Different letters between assessment days represent LSD test significant differences at p<0.05

The maize plant growth was differently depending on different individual maize hybrid treatment (Figure 4). At wilting point of the maize plant in the sixth assessment, the plants were in the same BBCH as in the fifth one. The percentage of the total germinated seeds from the treatments with both maize hybrid and water regime increased progressively during the experimental trial.

At the first assessment all the maize seeds reached BBCH 09 stage highlighting the emergence time, the cracking stage, the moment when coleoptile penetrates the soil surface and also the germination percentage. At the second assessment the maize seedlings had the highest share of 47% from the total germinated seeds were in the most advanced BBCH 12 stage for Inteligens hybrid under RHN. This hybrid had the most higher percentages until the end of the experiment regarding seedling development. The maize seedling had 3 unfolded leaves (BBCH 13 stage) at the third and fourth assessment dates.

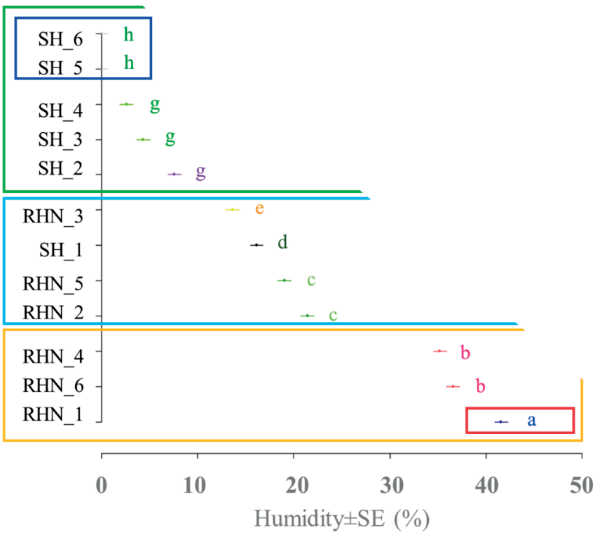

Figure 3. Soil moisture content from RHN-normal hydric regime and SH-hydric stress-drought, at all six assessments independent on the maize hybrid

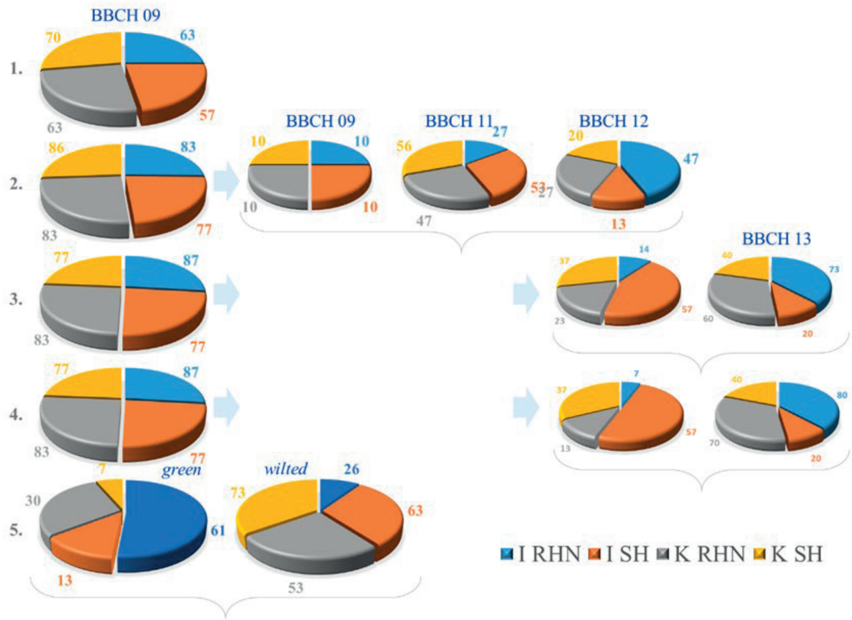

Figure 4. Maize hybrids development under RHN-normal hydric regime and SH-hydric stress-drought treatments on BBCH scale. First chart from all 5 assessments represents total germinated percentages independently on the secondary stages and the following charts represent seeds and seedlings in representative BBCH stage as percentages from the total germinated seeds-first chart. Inteligens maize hybrid - I, Kashmir maize hybrid - K

At the fourth assessment, the Inteligens maize hybrid from RHN treatment increased the percentage of seedlings in BBCH 13 stage with 17% compared with the third assessment. At the last assessment the green and wilted maize seedlings highlighted Inger in RHN more resilient compared to Kashmir hybrid in RHN and Kashmir less resilient to drought conditions with 10% more wilted plants compared to Inteligens hybrid in SH treatment respectively under drought conditions.

The two-way ANOVA revealed differences between leaf chlorophyll induced by the interaction of water regime and the assessments 1 (*p<0.01*), 5 and 6 (*p<0.001*) (Table 2). Neither the hybrid factor and the interaction between the hybrid and water regime did not determine significant differences between assessments regarding this parameter. The general trend is represented by a reduction of this parameter under drought conditions for both tested hybrids (Alvi et al., 2022; Jan et al., 2024). After 20 days of experimentation, drastic reduction was seen for Inteligens with 138 less SPAD value. For Kashmir were observed with 151 less SPAD units in drought conditions, with the statement that this hybrid recorded higher values for the treatments RHN with around 12% and SH with around 28% compared to Inteligens. Kashmir maize hybrid at the assessment 5 close to the wilting point the highest chlorophyll content of 170 SPAD units.

Table 2. Chlorophyll content difference between tested treatments to all six assessments

		C1	C2	C3	C4	C5	C6
I_RHN		216.18±19.27 a	151.5±4.48 a	212.59±29.91 a	147.69±1.04 a	152.32±5.39 a	121.96±9.44 a
I_SH		138.81±14.43 b	212.4±46.65 a	201.33±17.08 a	103.33±42.98 a	14.11±5.05 b	0±0 b
K_RHN		186.34±3.84 ab	176.87±4.81 a	143.29±12.86 a	141.13±9.08 a	169.91±20.21 a	81±22.42 a
K_SH		129.24±17.51 b	191.69±20.96 a	184.16±17.8 a	107.84±22.61 a	18.13±1.39 b	0±0 b
H	F	1.72	0.01	4.48	0.00	1.00	2.83
	p val	*0.226*	*0.930*	*0.067*	*0.968*	*0.345*	*0.131*
W	F	20.07	2.16	0.53	2.47	180.95	69.61
	p val	*0.002*	*0.180*	*0.489*	*0.155*	*<0.001*	*<0.001*
H:W	F	0.46	0.80	1.63	0.05	0.40	2.83
	p val	0.519	0.398	0.238	0.828	0.547	0.131

Note: Different letters indicate significant differences between treatments at each individual of all six assessments.
Legend: H - maize hybrid, W - water regime, RHN - normal hydric regime, SH - hydric stress, C - chlorophyll content 1-6- all six assessments, I - Inteligens maize hybrid, K - Kashmir maize hybrid.

Soil humidity highlights the significant changes depending on the interaction between maize hybrid and water regime (Table 3). The maize seedlings consumed from first assessment to the second 20% from soil humidity due to the leaf developmental stage. The BBCH 12 on which the plants were is part of the most important phenophases for maize when the water requirement is increased (***FAO). In the field conditions it is considered that this growth phase only use the water stored in the soil during winter time and maize crop does not need water from precipitation to emerge from soil and develop the first leaves (Jorda et al., 2022; Șimon et al., 2023). From the second to the third assessment, Inteligens hybrid needed 9% from the soil humidity and Kashmir 7% in RHN. This result highlights that Kashmir could use more efficiently the soil humidity however with a slow development compared to Inteligens. After wetting, at the fourth assessment the soil humidity had high values on Inteligens in RHN treatment had 80% from the total plants in BBCH 13 with three unfolded leaves at the fourth assessment, proving efficient soil water use (soil humidity 37%) (Figure 4). Kashmir in RHN had 70% from total plants in BBCH 13 with low soil humidity around 34%. This trend is suitable also for the fifth assessment of soil humidity. Here Inteligens hybrid from drought conditions still registered very low soil humidity percentage of 0.07% compared to Kashmir where the soil was completely dry.

Table 3. Soil humidity difference between tested treatments to all six assessments

		SH1	SH2	SH3	SH4	SH5	SH6
I_RHN		43.42±1.93 a	23.57±0.92 a	14.72±0.43 a	36.72±1.92 a	19.35±1.22 a	37.17±2.62 a
I_SH		15.77±1.91 b	7.1±1.19 b	3.82±0.68 b	2.65±0.81 b	0.07±0.07 b	0±0 b
K_RHN		39.73±0.63 a	19.33±2.28 a	12.63±1.49 a	33.72±2.74 a	18.72±1.73 a	36.03±1.27 a
K_SH		16.57±0.26 b	8.13±0.31 b	4.95±0.38 b	2.58±0.3 b	0±0 b	0±0 b
H	F	1.06	1.36	0.30	0.79	0.11	0.15
	p val	*0.333*	*0.278*	*0.599*	*0.401*	*0.750*	*0.707*
W	F	329.58	101.46	114.99	355.54	320.91	631.70
	p val	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
H:W	F	2.57	3.68	3.45	0.72	0.07	0.15
	p val	*0.148*	*0.091*	*0.101*	*0.421*	*0.796*	*0.707*

Note: Different letters indicate significant differences between treatments at each individual of all six assessments.
Legend: H - maize hybrid, W - water regime, RHN - normal hydric regime, SH - hydric stress, C - chlorophyll content 1-6- all six assessments, I - Inteligens maize hybrid, K - Kashmir maize hybrid.

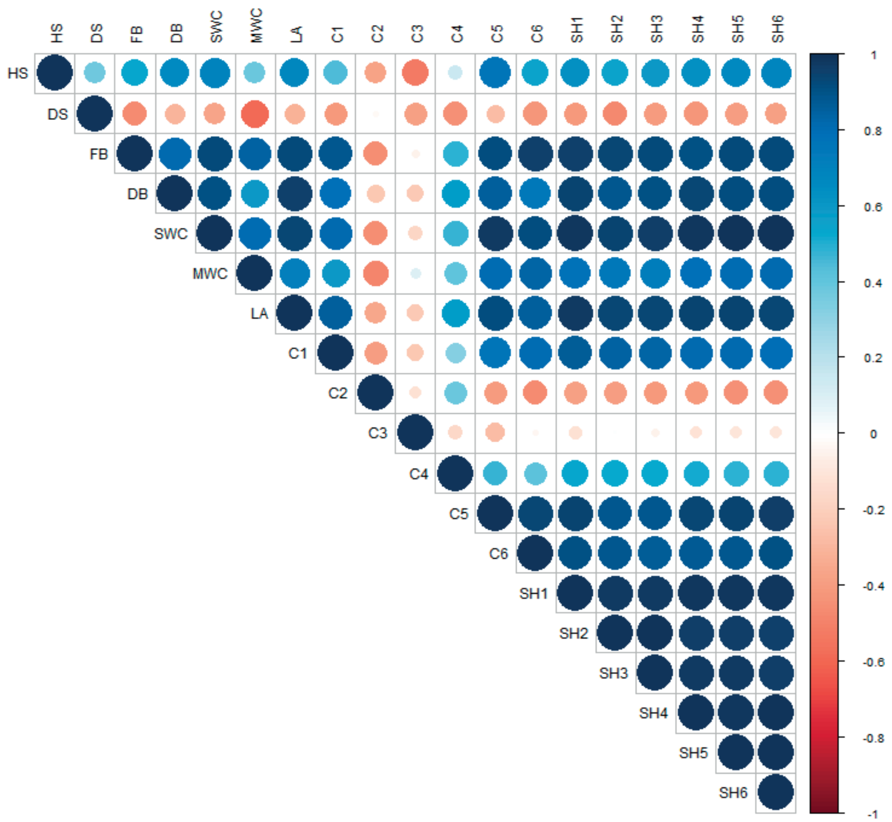

Figure 5. Correlogram chart between all analyzed parameters
Legend: The blue shades dots indicate positive correlations and the red shades dots indicate negative correlations between variables. HS, DS, FB, DB, SWC, MWC, LA, C1-C6, SH1-SH6

The correlation patterns between all registered and calculated values highlight both positive and negative effects with different strengths (Figure 5). Negative average correlation was observed between dry soil and fresh biomass, dry biomass, soil water content, maximum plant water content, leaf area, almost all assessments of chlorophyll content except the second one and all assessments of soil humidity. The chlorophyll content from first assessment and most of the morphological features are negatively correlated with the second and third evaluation. Morphological parameters assessed at the end of the experiment correlate strong and positively with chlorophyll content from the assessment 5 and 6 and with all six assessments regarding soil humidity.

CONCLUSIONS

Both hybrids endured drought conditions for up to 23 days from emergence to wilting.

The Kashmir hybrid exhibited higher water requirements and achieved the highest chlorophyll content. In contrast, the Inteligens hybrid was more resilient to water stress, showing the most advanced development on the BBCH scale, the largest accumulated biomass, and the most extensive leaf area.

The highest chlorophyll content was recorded in KWS Kashmir under normal water regime.

The most resistant to water stress conditions (drought) was the KWS Inteligens hybrid according to assessment 3 and 4 in terms of development on the BBCH scale.

The maximum leaf area was recorded in the KWS Inteligens hybrid.

Dry biomass is directly correlated with soil moisture; a higher water requirement was observed in KWS Kashmir compared to KWS Inteligens.

The assessment of water stress in corn cultivation in Romania is essential for ensuring sustainable agricultural production and for protecting the country's food security.

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